REMARKS

I. Status of the Claims

Claims 1, 4, 6, 8-27, 29-48 and 71 are pending and under consideration, claims 2, 3, 5, 7, 28 and 49-70 having been previously canceled without prejudice against their reintroduction into this or one or more timely filed continuation, divisional or continuation-in-part applications. With this Amendment, claims 1, 6, 8, 10, 15 and 27 are being amended. Thus, after entry of this Amendment, claims 1, 4, 6, 8-27, 29-48 and 71 remain pending and under consideration. The amendments of the claims and the various rejections raised in the Office Action are discussed in more detail, below.

II. Amendments

Claim 1 is amended to specify a method for improving the efficiency of transferring a nucleic acid into a plant cell having an intact cell wall, to move the depressurization element to step a), and to specify step c), transferring the nucleic acid into the plant cell using electroporation. Claim 27 is amended to specify a method for improving the efficiency of introducing a nucleic acid into a cell of a plant, wherein the cell has a cell wall, to move the depressurization element to step a), and to state that the introduction of the nucleic acid into the cell is by electroporation. Basis for the amendments of these claims can be found at least at paragraphs [0019], [0086], [0088] and [0109] of the published US Application 20060188992.

Claims 6, 8, 10 and 15 are amended for proper dependency.

No new matter is added by way of these amendments.

III. Rejection under 35 U.S.C. §112, second paragraph

Claims 1, 4, 6, 8-27, 29-48 and 71 were rejected under 35 U.S.C. §112, second paragraph as allegedly indefinite for failing to particularly point out and distinctly claim the subject matter which the applicant regards as the invention.

The amendments of claims 1, 6, 8, 10, 15 and 27 obviate the rejections.

Applicants respectfully request withdrawal of the rejection under 35 U.S.C. §112, second paragraph.

IV. Rejections under 35 U.S.C. §103

Claims 1, 4, 6, 8-27, 29-48 and 71 were rejected under 35 U.S.C. §103 as allegedly obvious over Dodgson (of record) in view of Gutierrez-Armenta *et al.* (of record) and Dev *et al.* (U.S. Patent 5,859,327).

Claims 1, 4, 6, 8-27, 29-48 and 71 were rejected under 35 U.S.C. §103 as allegedly obvious over Schmukler (of record) in view of Gutierrez-Armenta and Dev.

Claims 1, 4, 6, 8-27, 29-48 and 71 were rejected under 35 U.S.C. §103 as allegedly obvious over Rickwood (of record) in view of Gutierrez-Armenta and Dev.

These rejections are respectfully traversed.

A. The Present Claims

As amended, the present claims refer to a method for improving the efficiency of transfer of a nucleic acid into a plant cell having an intact cell wall, comprising the steps of: a) holding the cell under a pressure different from an atmospheric pressure wherein the pressure is reduced by about 0.096 MPa from the atmospheric pressure; b) placing the cell and the nucleic acid under conditions to induce electroporation and c) transferring the nucleic acid into the plant cell using electroporation (Claim 1); and a method for improving the efficiency of introducing a nucleic acid into a cell of a plant, wherein the cell has a cell wall, comprising the steps of: a) holding the cell under a pressure different from an atmospheric pressure wherein the pressure is reduced by about 0.096 MPa from the atmospheric pressure; b) placing the cell and the nucleic acid under conditions to induce electroporation and introducing the nucleic acid into the cell using electroporation; and c) differentiating, growing, and/or multiplying the cell (Claim 27).

B. The Cited Art

DODGSON discloses an apparatus and method for electroporation in which the apparatus may include one or more channels, and depressurization may be used to **position** cells in the channel(s) of the apparatus.

SCHMUKLER discloses an apparatus and method for electroporation and electrofusion of cells, in particular myeloma and lymphoma cells, which are types of animal cells, as well as isolated nuclei (see Col . 3 of Schmukler).

RICKWOOD discloses a method of transfecting cells involving the generation of bubbles of gas in a liquid medium which interact with the cell to be transformed and form a hole in the cell's surface.

GUTIERREZ-ARMENTA discloses the use of retinoblastoma protein to control growth of plant cells and/or plant viruses. Several methods of administering nucleotides to cells are disclosed, including electroporation of plant seed cells with DNA.

DEV discloses a method for producing genetically modified plants via electroporation in the absence of cell-wall degrading enzymes.

C. Analysis

C1. Legal Standard for Determining Obviousness Under 35 U.S.C. § 103(a)

Determining obviousness under 35 U.S.C. § 103(a) requires an objective analysis involving four factual inquiries, which include:

(a) determining the scope and content of the prior art,

(b) ascertaining the differences between the prior art and the claims at issue;

(c) resolving the level of ordinary skill in the art; and

(d) evaluating evidence of secondary considerations.

See Graham v. John Deere, 383 US 17, 18, 148 USPQ 459, 467 (1966); *see also* M.P.E.P. § 2141. A claim composed of several elements is not proved obvious merely by demonstrating that each of its elements was, independently, known in the prior art. *See KSR International Co. v. Teleflex Inc.*, 82 USPQ2d 1385, 1385 (US 2007). It is also important to identify a reason that would have prompted a person of ordinary skill in the relevant field to combine the elements in the way the claimed new invention does. *See id.* Thus, in assessing the scope and content of the prior art, the references must be considered in their entirety, *i.e.*, each as a whole including portions that would lead away from the claimed invention. *See W.L. Gore & Associates, Inc. v. Garlock, Inc.*, 721 F.2d 1540, 220 USPQ 303 (Fed. Cir. 1983), *cert. denied*, 469 US 851 (1984); *see also* M.P.E.P. § 2141.02.

C2. Rejection of claims 1, 4, 6, 8-27, 29-48 and 71 as allegedly obvious over Dodgson (of record) in view of Gutierrez-Armenta *et al.* (of record) and Dev *et al.* (U.S. Patent 5,859,327).

According to the Patent Office, Dodgson *et al.* teach a method for transferring a nucleic acid into a cell, that pressure can be applied to the cells to be transfected, and that a microprocessor can be used to vary system parameters, including pumping pressure, and that, given the control on the pumping pressure, depressurization is taught by the reference (see Office Action at page 4) and that a suitable choice of the voltage to be applied is a mere matter of optimization (Office Action at page 6).

Applicants disagree, and respectfully remind the Examiner that a Graham-type analysis requires that the reference be taken as a whole. The scope and content of the cited references clearly differs from the claimed subject matter. The primary reference, Dodgson *et al.*, teaches an apparatus and method for electroporation in which the apparatus may include one or more channels, and depressurization may be used to **position** cells in the channel(s) of the apparatus. Dodgson *et al.* neither shows nor suggests depressurization in which the plant cell is held under a pressure different from an atmospheric pressure wherein the pressure is reduced by about 0.096 MPa from the atmospheric pressure for electroporation. The Examiner's citation of page 14, line 20 through page 15, line 2 of Dodgson is completely misinterpreted and mischaracterized in the Office Action of 9 July 2008. A careful reading of page 4, lines 5-12, as well as page 14, line 20 through page 15, line 15, reveals that the disclosed pressure differential between two channels is clearly for the purpose of "localizing" (i.e., positioning) the cell in the channel, and the only reference to a pressure drop is at page 15, line 1, in which the pressure on the flow through the two channels drops from channel 4 to channel 52 in Figure 4e which allows the cell to move into position in the well 50. This is a pressure gradient causing a flow or localization of the cell to the well. There is no mention of negative pressure in the well holding the cell. In fact, at page 15, lines 3-5, a positive pressure is implied by the description of "physical force from a plunger or deformable section in the top wall 55 of channel 4." Also at page 15, lines 9-10, the specification states that electrodes may be provided at the orifice of the channel reducing the need for a pressure pulse. Thus, contrary to the Examiner's assertion that "it would have been an obvious matter of optimization on the part of the artisan of ordinary skill, particularly since Dodgson et al. teaches varying the pressure applied" (Office Action at page 4), in Dodgson, the need for pressure is not linked to the electroporation step, the specification teaches away from the need for pressure in conjunction with electroporation by teaching that electrodes may reduce the need for a pressure pulse, and positive pressure is disclosed rather

than depressurization, as required by the present claims. In contrast, Applicants specification clearly sets forth specific Examples and Figures involving a depressurization step is performed under a pressure reduced by about 0.096 MPa from the atmospheric pressure (see at least original claim 5, and Figures 2, 9, 20 and 21 of the application as filed).

Furthermore, as acknowledged by the Examiner, plant cells are not explicitly described. Moreover, plant cells are not implied or suggested, and would not work in the invention of Dodgson. This is clear from the description of the size of the channel, which is disclosed to be 50 μm, preferably less than about 25 μm (page 2, lines 8-9), which is large enough to serve as a conduit for a cultured animal cell, but not large enough to allow passage of a plant cell through to the well. Thus, one of skill in the art would have to completely change the channel conduction system as the operation of the apparatus of Dodgson to arrive at the presently claimed subject matter of transferring a nucleic acid into a plant cell.

Of the secondary references, Gutierrez-Armenta *et al.* disclose a plant-derived cDNA encoding retinoblastoma protein for controlling growth of plant cells and/or plant viruses. The Examiner cites paragraph [0015] of Gutierrez-Armenta which discloses electroporation of DNA as a possible method of indirect transformation as opposed to direct transformation using an expression vector. The Gutierrez-Armenta reference fails to cure the deficiency of the primary reference, as it, too, fails to teach or suggest depressurization in which the plant cell is held at a pressure reduced by about 0.096 MPa from the atmospheric pressure. Furthermore, the reference presents no working examples demonstrating successful introduction via electroporation of nucleic acids into plant cells using electroporation. Clearly this reference does not enable the skilled artisan to perform the presently claimed method.

Likewise, while the Dev reference discloses a method for producing genetically modified plants via electroporation in the absence of cell-wall degrading enzymes, it fails to teach depressurization in which the plant cell is held under a pressure different from an atmospheric pressure wherein the pressure is reduced by about 0.096 MPa from the atmospheric pressure for electroporation.

Thus, contrary to the assertion of the Examiner, the combination of the Dodgson, Gutierrez-Armenta and Dev references does not teach all the elements, nor does the combination lead the skilled artisan to select the specific depressurization conditions in which the plant cell is held at a pressure reduced by about 0.096 MPa from the atmospheric pressure as presently claimed.

C3. Rejection of claims 1, 4, 6, 8-27, 29-48 and 71 as allegedly obvious over Schmukler (of record) in view of Gutierrez-Armenta and Dev.

According to the Patent Office, Schmukler teaches a method of electroporation in which cells are trapped into pores in a film with diameters smaller than the diameters of the cells, and an electric field is applied to cause electroporation (Office Action at page 6). The Examiner also points to column 3, lines 44-47, which teaches a pressure gradient across the film containing the pores (Office Action at page 7).

Again, the reference must be taken as a whole. When considering the combination of references cited by the Examiner in making this rejection, the primary reference, Schmukler, clearly differs from the claimed subject matter in scope and content. The Schmukler reference discloses an apparatus and method directed to electroporation and electrofusion of cells, in particular myeloma and lymphoma cells (types of animal cells), as well as isolated nuclei (see Col . 3 of Schmukler). Thus, like Dodgson, Schmukler is directed to animal cells rather than plant cells, and fails to teach or suggest introduction of nucleic acids into plant cells. In fact, as judged by the working examples, Schmukler is largely focused on a method for fusing two types of cells, or fusing a cell with an isolated nucleus, rather than electroporation. Moreover, and again like Dodgson, Schmukler describes a negative pressure gradient for movement of a cell through a pore of a film. Schmukler does not teach or suggest a method in which a plant cell is held at a pressure reduced by about 0.096 MPa from the atmospheric pressure for electroporation.

The secondary references in this rejection, Gutierrez-Armenta and Dev are discussed above, and fail to cure the deficiencies of the Schmukler reference.

C4. Rejection of claims 1, 4, 6, 8-27, 29-48 and 71 allegedly obvious over Rickwood (of record) in view of Gutierrez-Armenta and Dev.

According to the Patent Office, Rickwood discloses a method of introducing a substance into a cell in which bubbles containing gas interact with the cell surface to form a hole in the surface of the cell, the substance introduced can be a nucleic acid, and the transfection can occur at a pressure below atmospheric pressure (Office Action at page 9).

Again, the scope and content of the cited references clearly differs from the claimed subject matter. The primary reference, Rickwood, teaches a method of transfecting cells using gas bubbles which interact with the cell to be transformed and form a hole in the cell's surface. The passage cited by the Examiner directed to depressurization involves the formation of the gas bubbles by "reducing the pressure to which the liquid medium is exposed, such that the solubility of the dissolved gas is reduced, causing the formation of bubbles in the liquid" (see

page 3 last paragraph bridging to page 4). This is clearly not the presently claimed method in which a plant cell is held at a pressure reduced by about 0.096 MPa from the atmospheric pressure for electroporation.

Furthermore, as previously argued, one atmospheric pressure is defined in Applicants' specification at paragraph [0105] of the published application as "typically, 1 atmospheric pressure=101.325 kPa=about 0.1 MPa." Thus, 0.1 MPa minus 0.096 MPa equals 0.004 MPa (4 x 10^3 Pa). In the last paragraph of page 6, where Rickwood discloses that transfection can be carried out under widely varying conditions, and specifies a range of atmospheric pressures, the range does not include the reduced atmospheric pressure required by the current claims. The limits of Rickman's disclosed range are from 1 x 10^4 Pa to 1 x 10^5 Pa, which encompasses a greater range of atmospheric pressures than the depressurized state required by the present claims in which the cell is held under a pressure reduced by about 0.096 MPa from the atmospheric pressure (approximately 4 x 10^3 Pa). Thus, Rickman does not teach or suggest the method as presently disclosed. Furthermore, although Rickwood does generally suggest that the disclosed method can be applied to cells having a cell wall, such as plant cells, fungal cells and bacteria (page 9), it is clearly stated that "(i)n this latter embodiment, it is preferred that the method is carried out on a protoplast derived from the cell." Clearly, such a method involving bubbles interacting with a cell surface to form holes in the cell surface would not work with cells having an intact cell wall.

The secondary references in this rejection, Gutierrez-Armenta and Dev are discussed above, and supply nothing to cure the deficiencies of the Rickwood reference.

In sum, none of the references, when considered either singly or in combination, teaches or suggests the presently claimed method for improving the efficiency of transfer of a nucleic acid into a plant cell having an intact cell wall, comprising the steps of: a) holding the cell under a pressure different from an atmospheric pressure wherein the pressure is reduced by about 0.096 MPa from the atmospheric pressure; b) placing the cell and the nucleic acid under conditions to induce electroporation and c) transferring the nucleic acid into the plant cell using electroporation (Claim 1); and a method for improving the efficiency of introducing a nucleic acid into a cell of a plant, wherein the cell has a cell wall, comprising the steps of: a) holding the cell under a pressure different from an atmospheric pressure wherein the pressure is reduced by about 0.096 MPa from the atmospheric pressure; b) placing the cell and the nucleic acid under conditions to induce electroporation and introducing the nucleic acid into the cell using electroporation; and c)

differentiating, growing, and/or multiplying the cell (Claim 27), nor is there any teaching or suggestion in the cited references that would lead one skilled in the art to modify the references to arrive at the presently claimed method.

Because the references, alone or in combination, fail to teach all the limitations of the present claims, the standard for obviousness has not been met. Accordingly, Applicants respectfully request withdrawal of the rejection under 35 U.S.C. §103.

CONCLUSION

In view of the foregoing, claims 1, 4, 6, 8-27, 29-48 and 71 are believed to satisfy all of the criteria for patentability and are in condition for Allowance. An early indication of the same is therefore kindly requested.

No fees other than the RCE fee are believed to be due in connection with this Amendment. However, the Commissioner is authorized to charge any additional fees that may be required, or credit any overpayment, to King & Spalding LLP Deposit Account No. 50-4616.

If, in the opinion of the Examiner, a telephone conference would expedite the prosecution of the subject application, the Examiner is encouraged to call the undersigned at (650) 590-1932.

Respectfully submitted,
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